UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

Note to Reader

Background: As part of its effort to involve the public in the implementation of the Food Quality Protection Act of 1996 (FQPA), which is designed to ensure that the United States continues to have the safest and most abundant food supply. EPA is undertaking an effort to open public dockets on the organophosphate pesticides. These dockets will make available to all interested parties documents that were developed as part of the U.S. Environmental Protection Agency's process for making reregistration eligibility decisions and tolerance reassessments consistent with FQPA. The dockets include preliminary health assessments and, where available, ecological risk assessments conducted by EPA, rebuttals or corrections to the risk assessments submitted by chemical registrants, and the Agency's response to the registrants' submissions.

The analyses contained in this docket are preliminary in nature and represent the information available to EPA at the time they were prepared. Additional information may have been submitted to EPA which has not yet been incorporated into these analyses, and registrants or others may be developing relevant information. It's common and appropriate that new information and analyses will be used to revise and refine the evaluations contained in these dockets to make them more comprehensive and realistic. The Agency cautions against premature conclusions based on these preliminary assessments and against any use of information contained in these documents out of their full context. Throughout this process, If unacceptable risks are identified, EPA will act to reduce or eliminate the risks.

There is a 60 day comment period in which the public and all interested parties are invited to submit comments on the information in this docket. Comments should directly relate to this organophosphate and to the information and issues available in the information docket. Once the comment period closes, EPA will review all comments and revise the risk assessments, as necessary.

These preliminary risk assessments represent an early stage in the process by which EPA is evaluating the regulatory requirements applicable to existing pesticides. Through this opportunity for notice and comment, the Agency hopes to advance the openness and scientific soundness underpinning its decisions. This process is designed to assure that America continues to enjoy the safest and most abundant food supply. Through implementation of EPA's tolerance reassessment program under the Food Quality Protection Act, the food supply will become even safer. Leading health experts recommend that all people eat a wide variety of foods, including at least five servings of fruits and vegetables a day.

Note: This sheet is provided to help the reader understand how refined and developed the pesticide file is as of the date prepared, what if any changes have occurred recently, and what new information, if any, is expected to be included in the analysis before decisions are made. **It is not meant to be a summary of all current information regarding the chemical.** Rather, the sheet provides some context to better understand the substantive material in the docket (RED chapters, registrant rebuttals, Agency responses to rebuttals, etc.) for this pesticide.

Further, in some cases, differences may be noted between the RED chapters and the Agency's comprehensive reports on the hazard identification information and safety factors for all organophosphates. In these cases, information in the comprehensive reports is the most current and will, barring the submission of more data that the Agency finds useful, be used in the risk assessments.

Jack E. Housenger, Acting Director
Special Review and Reregistration Division

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TOXIC SUBSTANCES

DATE: April 27, 2000

MEMORANDUM

SUBJECT: ***MALATHION****:* The Toxicology Chapter for the RED

FROM: Yung G. Yang, Ph.D.
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THROUGH: Alberto Protzel, Ph.D.
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TO: Paula A. Deschamp
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Health Effects Division (7509C)

DP Barcode: D265266	**Case: 818961**
Submission: S529758	**Chemical: Malathion**
PC Code: 057701	**Registrant: Cheminova Agro**

Action Requested: Prepare a revised toxicology chapter for the malathion RED.

Response: The following toxicology chapter for malathion was originally written by Dr. Brian Dementi (D244091, 3/24/98) and has been revised by Dr. Yung Yang to include the latest Cancer Assessment Review Committee's conclusions (meeting date: April 12, 2000).

HAZARD CHARACTERIZATION

1.0 HAZARD ASSESSMENT

The toxicity database for malathion is substantially complete and of acceptable quality to assess the potential hazard to humans, including special sensitivity of infants and children. The database will support a reregistration eligibility determination for the currently registered uses. However, two new toxicity studies have been required to fully comply with guideline requirements and to provide better hazard characterization: 1) a 90-day feeding study in dogs because the available 1-year study is unacceptable, and 2) a 90-day inhalation study in rats based on the results of the two-week range-finding study (MRID 44554301) and the lack of a NOAEL for cholinesterase inhibition in the 90-day study (MRID43266601). In addition, the Agency has recently issued FR42945 (August 6, 1999) requiring registrants of neurotoxic pesticides to conduct acute, subchronic, and developmental neurotoxicity studies. Thus, a developmental neurotoxicity study for malathion is required under this Data Call-in program.

Malathion is an organophosphorus (OP) insecticide, and like all members of this class, the mode of toxic action is the inhibition of cholinesterase (ChE). However, relative to other OP insecticides, malathion exhibits low acute oral toxicity in tests with technical material; and, unlike other OPs where acute dietary NOAELs have been established based on cholinesterase inhibition, the acute dietary NOAEL for malathion is selected from a compilation (synthesized) of studies and is considered to be conservative for a single exposure (acute) dietary risk assessment. With this exception, all other endpoints selected for malathion risk assessment were based on cholinesterease inhibition.

Results from developmental toxicity studies in rats and rabbits and a reproduction study in rats indicated that malathion does not cause developmental or reproductive toxicity. The data also demonstrated that there is no increased sensitivity of rats or rabbits to in utero or early post-natal exposure to malathion.

Malathion has been reviewed extensively by the Cancer Asessment Review Committee and is classified as **‘suggestive evidence of carcinogenicity but not sufficient to assess human carcinogenic potential”** by all routes of exposure. This classification was based on the following factors: (i) occurrence of liver tumors in male and female B6C3F1 mice and in female Fischer 344 rats only at excessive doses (statistically significant and outside historical control); (ii) the presence of a few rare tumors, oral palate mucosa in females and nasal respiratory epithelium in male and female Fischer 344 rats. With the exception of one nasal and one oral tumor in female rats, all other tumor types were determined to occur at excessive doses or were unrelated to treatment with malathion. These tumors can not be distinguished as either treatment related or due to random occurrence; (iii) the evidence for mutagenicity is not supportive of a mutagenic concern in carcinogenicity; and (iv) malaoxon, a structurally related chemical, is not carcinogenic in male or female Fischer 344 rats.

Malathion

A metabolism study in the rat indicated that malathion and its metabolites are excreted primarily in the urine (80-90%) in the first 24 hours following exposure, with lesser amounts excreted in the feces. At 72 hours, the highest concentration of radioactivity was observed in the liver, but less than 0.3% of the administered radioactivity was present in that organ. Radioactivity did not bioaccumulate in any of the organ/tissues analyzed. Although eight radiolabeled metabolites were observed in urine, greater than 80% of the radioactivity in urine was represented by the diacid (DCA) and monoacid (MCA) metabolites. The remaining radiolabeled metabolites were identified as components of “peak A” and “peak B”. It was determined that between 4 and 6% of the administered dose was converted to malaoxon, the active cholinesterase inhibiting metabolite of malathion.

A toxicity profile for malathion is as follows.

Toxicity Profile of Malathion Technical

Guideline	MRID#	Type of Study	Results	Tox. Cat.	Core Grade
Acute Toxicity					
§81-1 870.1100	00159876 (1986)	Acute Oral-Rat	LD_{50}= 5400(M)/5700(F) mg/kg	4	Acceptable
§81-2 870.1200	00159877 (1986)	Acute Dermal- Rat	LD_{50} >2000 mg/kg (M)(F)	3	Acceptable
§81-3 870.1300	00159878 (1986)	Acute Inhalation-Rat	LC_{50}> 5.2 mg/L (M)(F)	4	Acceptable
§81-4 870.2400	00159880 (1985)	Eye Irritation-Rabbit	Slight conjunctival irritation; Clear by 7 days	3	Acceptable
§81-5 870.2500	00159879 (1985)	Skin Irritation-Rabbit	Slight dermal irritation (PIS=1.1)	4	Acceptable
§81-6 870.2600	00159881 (1986)	Dermal Sensitization-Guinea pig	Not a skin sensitizer	N/A	Acceptable
Subchronic Toxicity					
§82-1(a) 870.3100	N/A	90-day feeding-Rat	N/A		Waived
§82-1(a) 870.3100	N/A	90-day feeding-Mouse	N/A		Waived
§82-1(b) 870.3150	N/A	90-day feeding-Dog	N/A		**Data Gap**
§82-2 870.3200	41054201 (1988)	21-day dermal- Rat (Malathion technical 94% a.i.)	ChEI NOAEL: 50 mg/kg/day ChEI LOAEL: 300 mg/kg/day, based on plasma and RBC cholinesterase inhibition in males; and plasma, RBC, and brain cholinesterase inhibition in females.		Acceptable
§82-3 870.3465	43266601 (1994)	90-day Inhalation- Rat (Malathion technical 96.4% a.i.)	Systemic NOAEL: not established Systemic LOAEL: 0.1 mg/L (LDT), based on histopathologic lesions of the nasal cavity and larnyx in males and females. ChEI NOAEL: not established ChEI LOAEL: 0.1 mg/L (LDT), based on plasma and RBC cholinesterase inhibition in females		Acceptable/ (nonguideline)
Chronic Toxicity					
§83-1 870.4100	40188501	Chronic toxicity-dogs Dose level:0,62.5,125,250 mg/kg/day	Systemic NOAEL: >250 mg/kg/day (HDT) ChEI NOAEL: Not established. ChEI LOAEL: <62.5 mg/kg/day based on plasma and RBC ChE inhibition		**Unacceptable**

Malathion

§83-2(b) 870.4200	43407201 (1994)	Carcinogenicity-B6C3F1 mice (Malathion technical 96.4% a.i.) Dose levels: 0, 100 ppm (17.4♂/20.8♀ mg/kg/d), 800 ppm (143♂/167♀ mg/kg/d), 8,000 ppm (1476♂/1707♀ mg/kg/d),16,000 ppm (2978♂/3448♀ mg/kg/d).	Systemic NOAEL: 143♂/167♀ mg/kg/day Systemic LOAEL: 1,476♂/1,707♀ mg/kg/day, based on decreased body weights and food consumption, increased liver weight, and increased hepatocellular hypertrophy in males and females. ChEI NOAEL: 17.4♂/20.8♀ mg/kg/day CHEI LOAEL: 143♂/167♀ mg/kg/day, based on plasma and RBC cholinesterase inhibition in males and females. Increased incidence of liver tumors in male and female mice only at excessive doses.	Acceptable
§83-5 870.4300	43942901 (1996)	Combined chronic toxicity/ carcinogenicity-F344 rats (Malathion technical 97.1% a.i.) Dose levels: 0, 50 ppm (2.4 mg/kg/d) 100/50 ppm (3.14♂/3.8♀ mg/kg/d), 500 ppm (26♂/32♀ mg/kg/d), 6,000 ppm (327♂/386♀ mg/kg/d), 12,000 ppm (677♂/817♀ mg/kg/d)	ChEI NOAEL: 2.4 mg/kg/day ChEI LOAEL: 29 mg/kg/day, based on significant plasma cholinesterase inhibition in males at 24 months. Increased incidence of liver tumors in female rats only at excessive doses.	Acceptable
Developmental / Reproductive Toxicity				
§83-3(a) 870.3700	41160901 (1989)	Developmental-Rat (Malathion technical 94% a.i.)	Maternal NOAEL: 400 mg/kg/day Maternal LOAEL: 800 mg/kg/day, based on reduced mean body weight gains and reduced mean food consumption. Developmental NOAEL: 800 mg/kg/day Developmental LOAEL: >800 mg/kg/day; no adverse developmental effects were observed at the highest tested dose.	Acceptable
§83-3(b) 870.3700	40812001 (1985)	Developmental-Rabbit (Malathion technical 92.4% a.i.)	Maternal NOAEL: 25 mg/kg/day Maternal LOAEL: 50 mg/kg/day, based on reduced mean body weight gains in does during the dosing period. Developmental NOAEL: 25 mg/kg/day Developmental LOAEL: 50 mg/kg/day based on a slightly increased incidence of mean resorption sites per dam.	Acceptable
§83-3(b) 870.3700	00152569 (1985)	Developmental Toxicity-Rabbit (range-finding) (Malathion technical 92.4% a.i.)	Maternal NOAEL: 100 mg/kg/day Maternal LOAEL: 200 mg/kg/day based on mortality and clinical signs of toxicity attributable to multiple doses. Developmental NOAEL: 400 mg/kg/day Developmental LOAEL: >400 mg/kg/day; upon external examination (only), no gross abnormalities were observed at the highest tested dose.	Acceptable
§83-4 870.3800	41583401 (1997)	Two-generation Reproduction-Rat (Malathion technical 94% a.i.)	Parental NOAEL: 394♂/451♀ mg/kg/day Parental LOAEL: 612♂ /703♀ mg/kg/day, based on decreased F0 generation body weights during gestation and lactation and decreased F1 pre-mating body weights. Offspring NOAEL: 131♂ /153♀ mg/kg/day Offspring LOAEL: 394♂ /451♀ mg/kg/day, based on decreased pup body weights during the late lactation period in F1 and F2 pups.	Acceptable

		Neurotoxicity		
§81-7 870.6100	40939301 (1988)	Acute Oral Delayed Neurotoxicity in the Hen (Malathion technical 93.6%)	Neither gross necropsies nor histopathological examination revealed any treatment-related effects in treated hens. Negative for any evidence of acute delayed neurotoxicity.	Acceptable
§81-8ss 870.6200	43146701 (1994)	Acute neurotoxicity-Rat (Malathion technical 96.4%)	NOAEL = 1000 mg/kg LOAEL = 2000 mg/kg (limit dose), based on decreased motor activity and clinical signs at the peak time of effect on day 1 (15 min post dosing) and plasma and RBC ChEI at day 7.	Acceptable
§82-7 870.6200	43269501	Subchronic neurotoxicity- Rat (Malathion technical 96.4%)	NOAEL (M/F): 4 mg/kg/day LOAEL (M/F): 352/395 mg/kg/day, based on plasma, RBC ChEI in males and females and brain ChEI in females. No neurotoxicity noted at high-dose.	Acceptable
		Mutagenicity		
§84-2 870.5100	40939302 (1987)	Gene mutation: *Salmonella typhimurium/ Escherichia coli*	**Negative** at all tested concentrations up to 5,000 μg/plate with and without S9 metabolic activation.	Acceptable
§84-2 870.5385	41451201 (1990)	Chromasome Aberration: *in vivo* bone marrow assay, rats	**Negative** in *in vivo* bone marrow cytogenetic assay at doses up to clinically and cytotologically toxic levels (2,000 mg/kg).	Acceptable
§84-2 870.5550	41389301 (1989)	Unscheduled DNA Synthesis Primary rat hepatocytes	**Negative** in *in vitro* primary rat hepatocytes for induction of UDS at doses up to cytotoxic levels (150-200 μg/mL).	Acceptable
		Metabolism		
§85-1 870.7485	41367701 (1989)	Metabolism-Rat	Malathion and its metabolites are excreted primarily in the urine (80-90%) in the first 24 hours following exposure, with lesser amounts excreted in the feces. At 72 hours, the highest concentration of radioactivity was observed in the liver, but less than 0.3% of the administered radioactivity was present in that organ. Radioactivity did not bioaccumulate in any of the organ/tissues analyzed. Although eight radiolabeled metabolites were observed in urine, greater than 80% of the radioactivity in urine was represented by the diacid (DCA) and monoacid (MCA) metabolites. The remaining radiolabeled metabolites were identified as components of “peak A” and “peak B”. It was determined that between 4 and 6% of the administered dose was converted to malaoxon, the active cholinesterase inhibiting metabolite of malathion.	Acceptable

2.0 DATA GAP(S)

1. 90-day feeding study in dogs (OPPTS 870.3150).
2. 90-day inhalation study in rats (OPPTS 870.3465)
3. **Developmental neurotoxicity study in rats (OPPTS 870.6300).

**The developmental neurotoxicity study is required under the Data Call-in program, FR42945, August 6, 1999.

3.0 HAZARD PROFILE

3.1 Acute Toxicity

The data base for acute toxicity is considered complete. Malathion exhibits low acute toxicity via the oral, dermal, and inhalation routes (Toxicity Category III or IV). It exhibits only slight eye and dermal irritation and is not dermally sensitizing.

For malaoxon (the cholinesterase-inhibiting metabolite of malathion), although no acute toxicity test data have been submitted, data are available from published literature (Dauterman and Main, 1966) that the acute oral LD_{50} for malaoxon is 158 mg/kg/day in rats. Based on a comparison of the malaoxon oral LD_{50} value from this study with the oral LD_{50} for malathion from a guideline study, malaoxon appears to have an acute oral toxicity that is approximately 10 to 30 times greater than that of malathion in rats.

3.2 Subchronic Toxicity

The requirements for subchronic feeding studies in the rodent and non-rodent (dog) were waived in the 1988 Malathion Registration Standard since chronic studies were imposed. However, the Hazard Identification Assessment Review Committee (HIARC) (1998) is requiring a 90-day study in the dog to address various deficiencies in the assessment of cholinesterase inhibition, including the absence of a NOAEL, in the chronic dog study. Also, HIARC (1998) determined that a new subchronic inhalation study is required based on the results of the two-week range-finding study (MRID 44554301) and the lack of a NOAEL for cholinesterase inhibition in the 90-day study (MRID 43266601).

In available subchronic studies with malathion, plasma and RBC cholinesterase inhibition were exhibited at the LOAEL in both rabbits and rats following dermal and inhalation exposure and brain cholinesterase inhibition in female rabbits following dermal exposure. Brain cholinesterase inhibition occurred at higher doses in both species. No clinical signs or other treatment-related effects were observed in dermally treated rabbits. Both clinical signs and treatment-related microscopic lesions of the nasal cavity and larnyx were observed in rats following inhalation exposure in whole body exposure chambers.

Executive summaries of 21-day dermal toxicity study in rabbits and 13-week inhalation study in rats are as follows.

In a **21-day dermal toxicity study in rabbits (MRID 41054201)**, groups of 6 male and 6 female New Zealand rabbits were treated dermally with undiluted technical malathion (94% a.i.) at dose levels of 0, 50, 300 or 1000 mg/kg/day for 6 hours/day, 5 days/week for 3 weeks. Assessments included clinical signs and mortality, dermal effects, food consumption, body weight, hematology and clinical chemistry (including cholinesterase activity of plasma, erythrocytes and brain). Gross necropsy was performed on all animals. The weight of the liver, kidneys, gonads and adrenals were recorded. Histopathology was performed on the following tissues for the high dose and control groups: adrenals, kidneys, liver, ovaries, skin (treated area), skin (mammary area), testes/epididymis and gross lesions.

With the exception of a dose-related decreased cholinesterase activity in both males and females at 1000 and 300 mg/kg/day, no treatment-related toxic effects (other than one possible mortality in the 1000 mg/kg/day group attributable to acute mucoid gastroenteritis) were observed in the study. No clinical signs of cholinesterase inhibition were noted. No treatment-related changes in body weights, food consumption, hematology, clinical chemistries, gross necropsies, organ weights or histopathology were observed. Dermal reactions at the application site were not observed. For males, the NOAEL and LOEL, respectively, for cholinesterase inhibition were considered to be the following: for plasma inhibition, 50 and 300 mg/kg/day (-13%); for RBC inhibition, 50 and 300 mg/kg/day (-18%); for brain (cerebrum) inhibition, 300 and 1000 mg/kg/day (-65%); and for brain (cerebellum) inhibition, 300 and 1000 mg/kg/day (-41%). For females, the comparable NOAELs and LOAELs were the following: for plasma inhibition, 50 and 300 mg/kg/day (-17%); for RBC inhibition, 50 and 300 mg/kg/day (-26%); for brain (cerebrum) inhibition, 50 and 300 mg/kg/day (-19%); and for brain (cerebellum) inhibition, 300 and 1000 mg/kg/day (-49%). For overall inhibition of cholinesterase activity in this study, the NOAEL was 50 mg/kg/day and the LOAEL was 300 mg/kg/day based on inhibition of plasma and RBC cholinesterase activity in males and females and on inhibition of brain (cerebrum) cholinesterase activity in females. The overall systemic NOAEL was 300 mg/kg/day and the overall systemic LOAEL was 1000 mg/kg/day based on possible mortality (1 male). This study is **ACCEPTABLE** and satisfies Guideline requirements.

In a **subchronic (13-week) inhalation study (MRID 43266601)**, groups of 15 male and 15 female Sprague-Dawley rats were exposed by inhalation in whole body exposure chambers to malathion (96.4% a.i.) aerosols (in air) at concentrations of 0, 0.1, 0.45 or 2.01 mg/L, 6 hours/day, 5 days/week for 13 weeks. The mass median aerodynamic diameters (MMAD) of the malathion particles were 1.6 μm at 0.1 mg/L and 1.7 μm at 0.45 and 2.01 mg/L. Assessments included those of clinical signs, body weight, food consumption, ophthalmoscopic examinations, hematology, clinical chemistry (including cholinesterase activity of plasma, erythrocytes and brain), urinalysis and gross and histopathology of Guideline required tissues.

Clinical signs such as urogenital staining, excess salivation and ungroomed fur were seen mostly at 2.01 mg/L, but occurred sporadically also at 0.45 and 0.1 mg/L in both sexes. After 13 weeks, dose-related decreases in cholinesterase activity were seen in both sexes. Relative to controls, cholinesterase activity decreases at 0.1, 0.45 or 2.01 mg/L, respectively were as follows: plasma 2%, 7% and 18% (males)and 16%, 30% and 70% (females); erythrocytes 9%, 22% and 43% (males) and 11%, 27% and 44% (females); brain 5%, 3% and 17%(males) and 4%, 8% and 41% (females). The review concluded that based on inhibition of erythrocyte and plasma cholinesterases exceeding 10% in female rats, the LOAEL was 0.1 mg/L and a NOAEL was not established. Microscopic lesions of the nasal cavity and larynx, classified as slight to moderate, were observed in most animals of both sexes at all three exposure concentrations. For this effect the LOAEL was 0.1 mg/L and the NOAEL could not be established. The study is acceptable/nonguideline because it was generally well conducted. However, it **DOES NOT SATISFY** Guideline requirements for a subchronic inhalation toxicity study in rats because a NOAEL was not established for plasma and erythrocyte cholinesterase inhibition in females, or for microscopic lesions of the nasal cavity and larynx in males and females.

3.3 Chronic Toxicity/Carcinogenicity

The available studies are adequate to satisfiy the chronic toxicity and carcinogenicity test requirement for malathion. An one-year feeding dog study was classified as unacceptable, however, the HIARC (1998) determined that a required subchronic oral dog study could possibly provide the required information.

Like other organophosphorus pesticides, the mode of toxic action for malathion is the inhibition of plasma, RBC, or brain cholinesterase (ChE) activity. Tumor incidences were observed in the liver, thyroid gland , testes, uterus, and mononuclear cell leukemia. The Cancer Assessment Review Committee (CARC) evaluated the carcinogenic potential of malathion and malaoxon (the active cholinesterase inhibiting metabolite of malathion) over a series of meetings during 1997-2000. Malathion is classified as **'suggestive evidence of carcinogenicity but not sufficient to assess human carcinogenic potential."** This classification was based on the following factors: (i) occurrence of liver tumors in male and female B6C3F1 mice and in female Fischer 344 rats only at excessive doses; (ii) the presence of a few rare tumors (oral palate mucosa - female and nasal respiratory epithelium - male and female) Fischer 344 rats. With the exception of one nasal and one oral tumor in female rats, all other tumor types were determined to occur at excessive doses or were unrelated to treatment with malathion. These tumors can not be distinguished as either treatment related or due to random occurrence; (iii) the evidence for mutagenicity is not supportive of a mutagenic concern in carcinogenicity; and (iv) malaoxon, a structurally related chemical, is not carcinogenic in male or female Fischer 344 rats.

Executive summaries of one-year feeding dog study, 18-month carcinogenicity study in mice, combined chronic toxicity/carcinogenicity study in rats, and combined chronic toxicity/carcinogenicity in rats with malaoxon (principal cholinesterase inhibiting metabolite of malathion) are as follows.

In an **one-year chronic oral toxicity study in dogs (MRID 40188501)**, malathion (95%) was administered daily in gelatin capsules to groups of 6 male and 6 female beagle dogs at dose levels of 0, 62.5, 125 or 250 mg/kg/day. There were no mortalities or treatment-related clinical signs of toxicity observed. No overall cholinesterase NOAEL was demonstrated in this study (<62.5 mg/kg/day). The overall cholinesterase LOAEL was 62.5 mg/kg/day (LDT) based on inhibition of plasma and erythrocyte cholinesterase activity in both males and females. The NOAEL for cholinesterase inhibition for both sexes was <62.5 mg/kg/day (LDT) for plasma and erythrocyte cholinesterase and 250 mg/kg/day for brain cholinesterase. The LOAEL for cholinesterase inhibition for both sexes was 62.5 mg/kg/day (LDT) for plasma and erythrocyte cholinesterase and >250 mg/kg/day (HDT) for brain cholinesterase. It was determined by the HIARC at one of its earlier meetings (9/9/97) that the systemic NOAEL in this study for both males and females was 250 mg/kg/day (HDT) and that no systemic LOAEL was demonstrated (>250 mg/kg/day). The study is **Unacceptable** and does not satisfy Guideline 83-1 for a chronic toxicity study in dogs because NOAELs were not established for inhibition of cholinesterase activity for plasma and erythrocytes in either males or females.

Although this study is classified as Unacceptable for the reasons given, HIARC (1998) determined that the deficiency could be addressed by a requirement of a 90-day study in dogs.

In a **combined chronic toxicity/carcinogenicity study in rats (MRID 43942901)**, malathion (97.1% a.i.) was administered to 90 Fischer 344 rats/sex/dose via the diet for up to 24 months at dose levels of 0, 100/50 (100 ppm for first 3 months of study, 50 ppm for duration of study in both sexes due to finding of erythrocyte cholinesterase inhibition in females only at 3 month assay) 500, 6,000 or 12,000 ppm [equivalent to respective mean values of 0, 4, 29, 359 and 739 mg/kg/day (males) and 0, 5, 35, 415 and 868 mg/kg/day (females)].

The only clinical sign observed was yellow anogenital staining among females at 12000 ppm. Increased mortality was seen in females at 12000 ppm and in males at 500, 6000 and 12000 ppm. All 12000 ppm males died or were sacrificed moribund by about 94 weeks. Treatment related decrements in body weight gain were observed at 6000 and 12000 ppm in both sexes. Food consumption was increased at 100 ppm in males for the first 3 months (prior to lowering of dose to 50 ppm). At subsequent time points for males, and across all time points for females food consumption was increased, the LOEL = 6000 ppm and NOEL = 500 ppm. Among parameters for hematology, erythrocyte count was reduced in males at 12000 ppm, mean corpuscular hemoglobin concentration was decreased in males at 6000 and 12000 ppm; and the following were observed in rats of both sexes at 6000 and 12000 ppm: increased platelet count, decreased mean corpuscular volume and mean corpuscular hemoglobin. Hence, for hematologic parameters overall, LOEL = 6000 ppm, NOEL = 500 ppm, both sexes. Among clinical chemistry parameters, erythrocyte cholinesterase inhibition, males, LOEL = 6000 ppm, NOEL = 500 ppm; females, at 3 months, the enzyme was inhibited at all doses, LOEL = 100 ppm. After 3 months, when lowest dose was reduced to 50 ppm, LOEL = 500 ppm, NOEL = 50 ppm. For plasma cholinesterase inhibition, males, LOEL = 500 ppm, NOEL = 50 ppm (100 ppm first 3 months); females, LOEL = 6000 ppm, NOEL = 500 ppm. For brain cholinesterase inhibition, LOEL = 6000 ppm, NOEL = 500 ppm, both sexes. For inhibition of cholinesterase activity, for males the overall NOEL is 50 ppm (4 mg/kg/day) and the LOEL is 500 ppm (29 mg/kg/day) based on inhibition of plasma activity at 24 months. For females the overall (beyond 3 months) NOEL is 50 ppm (5 mg/kg/day) and the LOEL is 500 ppm (35 mg/kg/day) based on inhibition of erythrocyte activity. Decreased aspartate aminotransferase, females, 12000 ppm; decreased alkaline phosphatase, males and females, 6000 and 12000 ppm; elevated blood urea nitrogen, males, 12000 ppm; elevated cholesterol, males and females, 6000 and 12000 ppm; elevated gamma-glutamyl transpeptidase, males and females, 6000 and 12000 ppm. Ocular effects testing inconclusive. Organ weight effects: increased kidney and liver weights, males and females, 6000 and 12000 ppm; thyroid/parathyroid weight increased (males), decreased (females) 6000 and 12000 ppm; increased spleen weight, males, 6000 and 12000 ppm; increased heart weight, males, 6000 ppm (term). In males, increases in liver and thyroid/parathyroid weights may have extended to 500 ppm. Microscopic findings: non-neoplastic: nasal mucosa and nasopharynx (several pathologies), males and females, 6000 and 12000 ppm; bilateral subacute-chronic inflammation/chronic nephropathy (high incidence in all study groups including controls), increased severity, males, 6000 and 12000 ppm, females, 500, 6000 and 12000 ppm; stomach (several pathologies), males and females, 6000 and 12000 ppm; increased incidence parathyroid hyperplasia, males and females, all doses; other findings in various tissues (thyroid, lymph nodes, lungs, liver, spleen, adrenal gland, eyes) as summarized in the review, being more remarkable in males, and often extending across the top three doses in males and top two doses in females; **neoplastic: treatment-related increased combined hepatocellular adenomas/carcinomas, females at all doses, incidences: 0/55 (0%), 2/55 (3.6%), 2/55 (3.6%), 3/55 (5.5%) and 6/55 (10.9%) for the 0, 100/50, 500, 6000 and 12000 ppm groups, respectively; rare tumors (one in each of four dose groups) on nasoturbinal slide preparations considered compound related effects: males, carcinoma 12000 ppm, adenoma 6000 ppm; females, squamous cell carcinoma 100/50 and 12000 ppm. Other tumor types observed included testes interstitial cell tumors significant at all doses with possibly decreased latency**; significant trend in thyroid follicular cell adenomas and/or carcinomas, males; significant trend and positive pairwise comparison at 500 ppm for thyroid c-cell carcinoma, males; significant difference in pair-wise comparison, mononuclear cell leukemia, 100/50 ppm, females; significant difference in pair-wise comparisons, pituitary pars distalis carcinomas, 500 and 6000 ppm, females; significant difference in pair-wise comparison, pituitary pars distalis adenomas and/or carcinomas combined, 500 ppm, females. Tumorigenic responses may have been compromised by high mortality in males at 6000 and 12000 ppm and in females at 12000 ppm. This chronic toxicity/carcinogenicity study in the F344 rat is classified as Acceptable.

On October 28, 1999, the HIARC was asked to reevaluate the mean compound intake because of the changes in the dose levels administered during the course of this study (i.e., 100 ppm for 1 to 16 weeks and 50 ppm for 18 to 102 weeks). The mean test substance intake for rats of both sexes at all doses has been recalculated using periodic test substance intake data from Table G-85 (pp. 482-493 of the study report, MRID 43942901). These calculations confirm that test compound intakes are actually somewhat lower than those cited in the DER from the study report. Based on the data, the NOAEL at 50 ppm should then be converted to 2.37 mg/kg/day for males and to 2.95 mg/kg/day for females (as opposed to 4 mg/kg/day and 5 mg/kg/day for males and females, respectively). (HIARC Report, November 1, 1999; HED Doc. 013820).

In an **<u>18-month carcinogenicity study in mice (MRID 43407201)</u>**, technical grade malathion (96.4% a.i.) was administered in the diet to groups of 65 male and 65 female B6C3F1 BR strain mice at dose levels of 0 (control), 100 ppm, 800 ppm, 8000 ppm or 16000 ppm (equivalent to 0, 17.4, 143, 1476 or 2978 mg/kg/day in males and to 0, 20.8, 167, 1707 or 3448 mg/kg/day in females). NOTE: The Agency required doses of 8000 and 16000 ppm be included in the study to address liver tumor response in males in the 1978 National Cancer Institute study.) Cholinesterase (plasma, erythrocyte and brain) activity was assayed at 9 (erythrocyte cholinesterase only), 12 and 18 months.

At 8000 ppm and 16000 ppm in both males and females, treatment related effects included decreased absolute body weights ranging from 14.3 to 20.0% in males and 9.7 to 16.1% in females throughout the entire duration of the study. Decreased food consumption was noted at 16000 ppm for mice of both sexes during the first 3 weeks and 13 weeks. After 26 weeks and for the remainder of the study, dose-related decreases in food consumption were observed at 8000 ppm and 16000 ppm, both sexes. Statistically significant inhibition of plasma and erythrocyte cholinesterase activity was observed in males at 8000 and 16000 ppm and in females at 800, 8000 and 16000 ppm, while inhibition of brain cholinesterase activity was seen in males and females only at 16000 ppm. Mortality rates, clinical signs of toxicity and hematological parameters were not affected by treatment with malathion at any dose.

A treatment-related increased incidence of hepatocellular tumors was observed in both male and female mice in this study at 8000 ppm and 16000 ppm. The percent incidences of hepatocellular adenomas for males were 1.9%, 7.3%, 3.6%, 21.8% and 94.1%; of hepatocellular carcinomas were 0.0%, 10.9%, 5.5%,%, 10.9% and 2.0%; and of combined hepatocellular adenomas/carcinomas were 1.9%, 18.2%, 9.1%, 32.7% and 96.1% for the 0 (control), 100, 800, 8000 and 16000 ppm groups, respectively. For male mice, combined incidences at 16000, 8000 and 100 ppm were statistically significant by pair-wise comparison and the dose trend was positive. For female mice, the percent incidences of hepatocellular adenomas were 0.0%, 1.8%, 0.0%, 17.0% and 80.8%; of hepatocellular carcinomas were 1.8%, 0.0%, 3.7%, 1.9% and 3.8%; and of combined hepatocellular adenomas/carcinomas were 1.8%, 1.8%, 3.7%, 18.9% and 84.6% for the 0 (control), 100, 800, 8000 and 16000 ppm groups, respectively. Combined incidences at 16000 and 8000 ppm were statistically significant by pair-wise comparison and the dose trend test was positive.

The increased tumor incidences in the livers of both males and females at 8000 ppm and 16000 ppm were accompanied by concurrent observations of masses, nodules and foci in the livers of these animals at the terminal sacrifice and also by increased liver weights and highly increased incidences of hepatocellular hypertrophy in the livers at 12 and 18 months. The data for hepatocyte hypertrophy was quite remarkable in that an extremely steep dose-response curve was observed for both males and females in this study. Thus, in the control, 100 ppm and 800 ppm groups, no case of hepatocellular hypertrophy was observed in any animal at any time during the entire duration of this study whereas at 8000 ppm and 16000 ppm, a >50% incidence was observed at 12 months and a 100% incidence at 18 months.

Other findings were observed in this study that appeared to be related to treatment, but their biological significance was uncertain. These findings included the following: decreased vacuolation in the convoluted tubules of the kidneys in males; increased mineralization of the kidneys in females; decreased fibrous osteodystrophy of the femur and sternum in females; and early disappearance of the "x zone" in the adrenal cortex of females.

The NOAEL for cholinesterase inhibition for both sexes was estimated to be 100 ppm (17.4 mg/kg/day in males and 20.8 mg/kg/day in females) for plasma and erythrocyte cholinesterases and 8000 ppm (1476 mg/kg/day in males and 1707 mg/kg/day in females) for brain cholinesterase. Although there was some decrease in cholinesterase activity at these doses, the decreases were not statistically significant and the data were considered to be too variable to conclude that the inhibition seen was really related to treatment. The LOAEL for cholinesterase inhibition for both sexes was estimated to be 800 ppm (143 mg/kg/day in males and 167 mg/kg/day in females) for plasma and erythrocyte cholinesterase and 16000 ppm (2978 mg/kg/day in males and 3448 mg/kg/day in females) for brain cholinesterase. The NOAEL for systemic effects was 800 ppm (143 mg/kg/day in males and 167 mg/kg/day in females). The LOAEL was 8000 ppm (1476 mg/kg/day in males and 1707 mg/kg/day in females), based on decreased body weights and food consumption in males and females, increased liver weight in males and females and increased hepatocellular hypertrophy in males and females. The study is classified Acceptable.

In a **combined chronic toxicity/carcinogenicity study (MRID 43975201),** malaoxon (96.4% a.i.), the cholinesterase inhibiting metabolite of malathion, was administered to F344 rats via the diet for up to 104-105 weeks at dose levels of 0, 20, 1000 or 2000 ppm (equivalent to 0, 1, 57 and 114 mg/kg/day in males and 0, 1, 68 and 141 mg/kg/day in females).

Ten animals/sex/group were sacrificed at 3, 6 and 12 months for interim evaluations and cholinesterase activity determinations. Standard parameters were examined. Full histopathological examinations were performed on control and high dose animals at 12 and 24 months and on all animals that died or were sacrificed during the study. Additional tissues, as appropriate, also were examined from other dose groups.

Mortality was significantly increased in high dose males (control, 29%; high dose, 53%) and in mid and high dose females (control, 13%; mid dose, 44%) high dose, 49%. Body weights were decreased in the high dose males and females throughout most of the study. The mean terminal body weight of high dose males was statistically significantly decreased by 14% compared to the control group. The mean terminal body weight of high dose females was decreased by 11% but did not reach statistical significance. Food intake was consistently greater in both sexes at the high dose and increased sporadically at the mid dose throughout the study. Treatment-related yellow anogenital staining was observed in high dose males and females. Increased incidences of emaciated rats were seen especially among the early decedent females.

Foreign material (food, hair) and cellular debris were found in the nasal cavity of high dose males and mid and high dose females. Nasal lumen inflammation was seen in high dose males and in mid and high dose females. Nasal lumen epithelial hyperplasia was increased in mid and high dose females. Lung interstitium inflammation was increased in mid and high dose females, and tympanic cavity inflammation was seen in mid and high dose early female decedents. Increased incidences of mineral deposits in the stomach muscularis were seen in mid and high dose males and females. The mean liver and kidney weights were increased in high dose males at 12 months, and the mean adrenal weight was increased in high dose males at 24 months. The mean spleen weight was decreased in high dose females at 24 months.

The plasma cholinesterase activity was decreased in males by 74%-91% and in females by 82%-96% compared to the controls after 3, 6, 12 and 24 months of malaoxon treatment at the mid and high doses. The erythrocyte cholinesterase activity was decreased 54-66% in makes and 45%-65% in females at the mid and high doses. The erythrocyte cholinesterase activity was also decreased by 21% in males and 19 % in females at 6 months of treatment at 20 ppm. Brain cholinesterase activity was decreased 11-18% during months 3-12 and 74% at 24 months compared to controls in high dose males and at the mid dose by 30% at 24 months. It was decreased by 61%-78% in high dose females at all time points and by 5%-14% at the mid dose ater 3, 6, and 12 months of treatment in females.

A NOAEL was not determined for cholinesterase activity inhibition in this study. The LOAEL is 20 ppm (1 mg/kg/day) for males and females based on the 19-21% inhibition of erythrocyte cholinesterase activity after 6 months of treatment. A NOAEL of 20 ppm (1 mg/kg/day) and a LOAEL of 1000 ppm (57 mg/kg/day for males, 68 mg/kg/day for females) for systemic toxicity were defined. In females, the systemic LOAEL was based on increased mortality, and microscopic changes in the LOAEL was based on increased mortality, and microscopic changes in the nasoturbinal tissues, lung interstitium, and tympanic cavity. In males, the systemic LOAEL was based on mineral deposits in the stomach muscularis.

The only statistically significant tumorigenic response was that of leukemia in male rats at the 2000 ppm dose level, accompanied by a positive dose-trend analysis, as derived from a statistical re-analysis of the data as performed by the registrant at the request of Health Effects Division (HED) subsequent to the initial review of the study. This particular re-analysis also found that when the high dose group was removed from statistical consideration, remaining pairwise comparisons were not statistically significant, but a marginally positive ($p = 0.054$) trend was observed. See memorandum of Carol Auletta of Huntingdon Life Sciences to Judy Hauswirth of Jellinek, Schwartz and Connolly, dated September 22, 1997 (MRID 44479301). It is noteworthy that the chronic toxicity/carcinogenicity study report (MRID 43975201) claims that "The statistical analysis of male testicular interstitial cell tumors indicated a difference in the time corrected incidence at the 2000 ppm dose level at the 0.03 level by Cox's test and at the 0.01 level by the Gehan-Breslow test. There was no difference in incidence at the 2000 ppm dose level by the Fishers exact test. The incidence of testicular interstitial cell tumors at 1000 ppm was statistical significant by the Fishers exact test. A positive trend test was obtained for this finding." (pp. 74-75) However, the study report discounted the findings on the grounds that this is a common tumor type in F344 rats and the incidences were within the expected range of NTP data and the historical control data base from the performing laboratory. This combined chronic toxicity/carcinogenicity study in the rat is classified **ACCEPTABLE**.

Summaries of carcinogenicity studies previously evaluated by HED's 1990 Cancer Peer Review Committee are presented below.

> The following summaries of 6 carcinogenicity studies are not intended to be full Executive Summaries, but rather to briefly summarize the major overall findings in the studies and to indicate the final disposition of the studies. All 6 studies were considered by the HED Carcinogenicity Peer Review Committee in 1990. One study, the malaoxon study on B6C3F1 mice, was considered to be acceptable and negative for carcinogenicity. The remaining 5 studies were determined by the committee to be inadequate to make a definitive determination of the carcinogenicity of malathion or malaoxon. Mostly on the basis of these studies, the committee classified malathion as a Group D carcinogen, (i.e., not classifiable as to human carcinogenicity). The committee also reaffirmed the requirements in the 1988 Registration Standard that the registrant be required to perform and submit a new chronic feeding study in F344 rats on malathion, a new carcinogenicity study in B6C3F1 mice on malathion and a new combined chronic feeding/carcinogenicity study in F344 rats on malaoxon. Unlike the Registration Standard, the committee required that the new chronic feeding study in rats on malathion be combined with a full carcinogenicity study. All 3 of these new studies have now been received and reviewed by the Agency. These new studies were discussed in pp 10-14 of this document.

Study 1 Malathion Carcinogenicity Study in Osborne-Mendel Rats.

Reference: MRID 00043268: National Cancer Institute (1978) See also Huff et al (1985) and McConnell (1984)

In a 113-week carcinogenicity study in Osborne-Mendel rats, technical grade malathion ($\geq$ 95% purity) was administered in the diet to groups of 50 male and 50 female rats at dose levels of 4700 ppm (time weighted average) and 8150 ppm (time weighted average) for 80 weeks. The rats were then observed for an additional 29-33 weeks. Control rats consisted of matched controls (15/sex) and of pooled controls (matched controls plus 40/sex from other contemporary bioassays at the same laboratory). All surviving rats were sacrificed at 109-113 weeks.

The original NCI report concluded "there was no clear evidence" of carcinogenicity in this study, although a statistically significant positive trend for thyroid follicular cell adenomas and carcinomas in female rats was noted. Subsequent to the original NCI report, a histopathological reexamination of the thyroid glands and adrenal glands of rats in this study was conducted by a panel of expert pathologists (NTP reevaluation). The NTP reevalaution diagnosed additional thyroid follicular cell adenomas in the control and low dose groups that eliminated the positive trend previously reported by NCI and concluded "there was no evidence of carcinogenicity" in this study. Subsequent to the NCI and NTP reports, an independent assessment of the results in this study was performed by the HED Carcinogenicity Peer Review committee. This committee noted aparent increases in thyroid C-cell adenomas/ carcinomas in males, in thyroid follicular cell adenomas/carcinomas in males and females, and in adrenal gland pheochromocytomas in males. **The committee also noted, however, numerous deficiencies in the design, evaluation and reporting of this study with respect to contemporary guidelines and concluded that this study was inadequate to make a definitive determination of the carcinogenicity of malathion.**

This study is **NOT ACCEPTABLE** and **DOES NOT SATISFY** guideline 83-2 for a carcinogenicity study in rats.

<u>Study 2</u> Malathion. Carcinogenicity Study in Fischer 344 Rats.

Reference: MRID 00043269: National Cancer Institute (1979) See also Huff et al (1985) and McConnell (1984)

In a 106-week carcinogenicity study in Fischer 344 rats, technical grade malathion (95% purity) was administered in the diet to groups of 49-50 male and 49-50 female rats at dose levels of 0 (matched control), 2000 or 4000 ppm for 103 weeks. The rats were sacrificed 2-3 weeks later at 105-106 weeks. Control rats were matched controls.

The original NCI report concluded "malathion was not carcinogenic in male or female rats, but the females may not have received a maximum tolerated dose." The NCI noted a statistically significant increase in adrenal gland pheochromocytomas in the low dose males (by pair-wise comparison), but did not consider this to be associated with the administration of malathion. A histopathological reexamination of the male adrenal glands was subsequently conducted by a panel of expert pathologists (NTP reevaluation). The NTP reevaluation suggested 2 types of neoplasms appeared to be marginally increased in the low dose males: adrenal gland pheochromocytomas and also leukemia. These increases were statistically significant by life-table analyses, but not by incidental tumor tests or pair-wise tests. The NTP indicated that life-table analyses are appropriate if the lesion is the cause of death, but it was judged that early deaths in this study were due to chemical toxicity. Hence, the NTP concluded "there was no evidence of carcinogenicity in male or female Fischer 344 rats" in this study. Subsequent to the NCI and NTP reports, an independent assessment of the results in this study was performed by the HED Carcinogenicity Peer Review Committee. This committee reaffirmed the apparent increases in adrenal gland pheochromocytomas and leukemias in the low dose males. The committee was unable, however, to perform an independent statistical evaluation of the data in the NTP report because insufficient individual animal data were presented in the report. High mortality in the malathion-treated males also confounded interpretation of the study results. Further, the highest dose level tested may not have been high enough in females. In addition, the committee noted several significant non-neoplastic findings in this study: stomach inflammation and ulceration in males, fatty metamorphosis and focal cellular changes in the liver of females, and chronic inflammatory changes in the kidneys of females. **Based on these considerations and certain additional deficiencies in the study with respect to contemporary guidelines, the committee concluded that this study was inadequate to make a definitive determination of the carcinogenicity of malathion.**

This study is **NOT ACCEPTABLE** and **DOES NOT SATISFY** guideline 83-2 for a carcinogenicity study in rats.

<u>Study 3</u> Malathion. Combined Chronic Toxicity/ Carcinogenicity Study in Sprague-Dawley Rats.

References: MRID 00110562: G. Rucci et al. (1980); MRID 41842401: J. C. Seely (1991)

In a 24-month combined chronic toxicity/carcinogenicity study in Sprague-Dawley rats, malathion (technical grade Cythion, 92.1% purity) was administered in the diet to groups of 50 male and 50 female rats at dose levels of 0, 100, 1000 or 5000 ppm. Surviving animals were sacrificed at 24 months.

Although this study was determined by the Agency to be unacceptable for use as a chronic toxicity study or a carcinogenicity study, an independent reevaluation of all the microscopic slides from this study, nevertheless, was required to be performed and submitted to the Agency. It was understood that submission of this reevaluation would not elevate the study to acceptable status because there were substantial faults in the design, conduct and reporting of this study. The initial review of this study indicated statistically significant increases in uterine polyps in females in the low and high dose groups, but no significant dose-related trend. Also in female rats, a statistically significant trend but no significant pair-wise differences were observed for thyroid C-cell tumors. Numerous non-neoplastic effects were also reported in the study. **In 1990, the HED Carcinogenicity Peer Review Committee concluded that "there should not be much weight put upon these findings" and that the study was insufficient to provide definitive evidence on the carcinogenicity of malathion.**

The independent reevaluation of all the microscopic slides from this study was submitted in 1991.

Malathion

The following is quoted from the memo reviewing the reevaluation (Dementi, 7/22/92): "Conclusion. The reevaluation did not disclose any remarkable adverse histopathologic effects of malathion other than chronic hepatotoxicity at the highest dose, thus allaying any concern that the original examination of this 1980 FDRL study failed to identify possible non-neoplastic or neoplastic effects of malathion beyond those indicated in that original examination. In fact, the reexamination reveals less evidence of a chronic toxicologic effect of malathion. A number of effects in the original examination which appeared to be occurring even at the lowest dose are not so characterized in the reevaluation. Toxicology Branch is concerned about the large disparity between these two examinations with respect to certain toxic end points." This study is **NOT ACCEPTABLE** and **DOES NOT SATISFY** Guideline 83-2 for a carcinogenicity study in rats.

Study 4 Malathion. Carcinogenicity Study in B6C3F1 Mice.

Reference: MRID 00043268: National Cancer Institute (1978)

In a 95-week carcinogenicity study in B6C3F1 mice (NCI, 1978), technical grade malathion ($\geq$ 95% purity) was administered in the diet to groups of 50 male and 50 female mice at dose levels of 8000 and 16000 ppm for 80 weeks. The mice were then observed for an additional 14-15 weeks. Control mice consisted of matched controls (10/sex) and of pooled controls (matched controls plus 40/sex from other contemporary bioassays at the same laboratory). All surviving mice were sacrificed at 94-95 weeks.

An increased incidence of hepatocellular carcinomas was observed in the male mice in this study. For combined neoplastic nodules and hepatocellular carcinomas, a statistically significant positive trend ($p = 0.019$) was noted; in addition, a p value of 0.031 (Fishers Exact Test) was observed when the high dose male group was compared to the pooled male control group. The NCI did not consider this to be a positive finding, however, because it employed as its criterion of significance a p value of ≤ 0.025, based on Bonferroni adjustments. The original NCI report, therefore, concluded there was "no clear evidence of carcinogenicity in this study. The NTP did not reevaluate the histopathological results in this study. The HED Carcinogenicity Peer Review Committee, when considering the results in this study, did reaffirm the increased incidence of hepatocellular tumors in the male mice and observed that a large proportion of these tumors was due to an increase in carcinomas. The committee was unable, however, to perform an independent statistical evaluation because individual animal data and detailed information about the pooled controls were not presented in the NCI report. **The committee also noted numerous deficiencies in the design, evaluation and reporting of this study with respect to contemporary guidelines and concluded that this study was inadequate to make a clear conclusion about the carcinogenicity of malathion.**

This study is **NOT ACCEPTABLE** and **DOES NOT SATISFY** guideline 83-2 for a carcinogenicity study in mice.

Malathion

Study 5 Malaoxon. Carcinogenicity Study in Fischer 344 Rats.

References: MRID 00043270: National Cancer Institute (1979) See also Huff et al (1985) and McConnell (1984)

In a 105-week carcinogenicity study in Fischer 344 rats, malaoxon (> 95% purity), the cholinesterase inhibiting metabolite of malathion, was administered in the diet to groups of 50 male and 50 female rats at dose levels of 0 (matched controls), 500 or 1000 ppm for 103 weeks. The rats were sacrificed up to 2 weeks later at 103-105 weeks. Control rats were matched controls.

The original NCI report noted a possible increase in thyroid C-cell adenomas or carcinomas in female rats at the high dose (11%). This finding was questioned, however, when the incidence was compared to the "historical" control data from the same laboratory (7%). The same NCI report also reported an increased incidence of benign mammary gland tumors in female rats at the low dose (p = 0.026), but did not consider this finding to be significant because it employed as its criterion of significance a p value of $\leq$ 0.025, based on Bonferroni adjustments. In addition, the original NCI report also noted an increase in the incidence of adrenal glands pheochromocytomas in males but determined these increases were not statistically significant. The original NCI report concluded that under the conditions of this study, malaoxon was not carcinogenic in fischer 344 rats.

Subsequent to the NCI report, a histopathological reexamination of the thyroid glands, mammary glands and adrenal glands of rats in this study was conducted by a panel of expert pathologists (NTP reevaluation). The NTP reevaluation, in contrast to the original NCI report, concluded there was "equivocal evidence" of carcinogenicity for both male and female rats in this study based on their findings for thyroid C-cell neoplasms. For females, the NTP reported an increased incidence of thyroid C-cell adenomas and carcinomas (23%) that was statistically significant (p = 0.045) at the high dose when compared to the female control group (8%) and yielded a statistically significant positive trend. For males, the NTP also reported an increased incidence of thyroid C-cell adenomas and carcinomas (20%) that was statistically significant (p = 0.035) at the high dose when compared to the male control group (6%) and yielded a statistically significant positive trend. The NTP also reaffirmed the increased incidence of benign mammary gland adenomas in the low dose females, but did not consider this increase to be treatment-related because it was not dose-related and the incidence in the concurrent controls was unusually low. The NTP count of adrenal gland pheochromocytomas in males differed considerably from the NCI count, but did not result in a statistically significant increase in these tumors in the malaoxon-treated males. The NTP report also noted, but did not discuss, an evident increase in the incidence of lymphomas (hemopoietic system) among high dose males. The NTP also reported in this study increased incidences of forestomach ulcers in males and females.

The HED Carcinogenicity Peer Review Committee, in its subsequent assessment of this study, agreed with the NTP finding of "equivocal evidence" of carcinogenicity based on the increased incidence of thyroid C-cell neoplasms in both males and females. The committee also was not able to totally dismiss concerns relating to the mammary gland adenomas in females, the adrenal gland pheochromocytomas in males, or the lymphomas in males. **Noting the lack of detailed data from which to perform an independent statistical analysis, the uncertainty of the total findings in the study, and other deficiencies and shortcomings in the study, the committee concluded that this study was inadequate to make a definitive determination of the carcinogenicity of Malaoxon in Fischer 344 rats.**

This study is **NOT ACCEPTABLE** and **DOES NOT SATISFY** guideline 83-2 for a carcinogenicity study in rats.

<u>Study 6</u> <u>Malaoxon</u>. Carcinogenicity Study in B6C3F1 Mice.

Reference: MRID 00043270: National Cancer Institute (1979)

In a 105-week carcinogenicity study in B6C3F1 mice (NCI, 1979), <u>malaoxon</u>, the cholinesterase inhibiting metabolite of malathion, was administered in the diet to groups of 50 male and 50 female mice at dose levels of 0 (matched controls), 500 or 1000 ppm for 103 weeks. The mice were sacrificed up to 2 weeks later at 103-105 weeks. Control mice were matched controls.

The original NCI report concluded that under the conditions of this study, malaoxon was not carcinogenic in B6C3F1 mice. The NTP did not evaluate the histopathological results in this study. **The HED Carcinogenicity Peer Review Committee which subsequently reviewed the findings in this study, concurred with the NCI report and concluded that in this study, <u>malaoxon</u> did not induce a treatment- related increase in tumors in B6C3F1 mice.**

This study is **ACCEPTABLE** and **SATISFIES** guideline 83-2 for a carcinogenicity study in mice. The above summaries of the earlier cancer studies taken from HED Tox Doc. No. 012433, prepared by Ed Budd, dated December 9, 1997.

3.4 Developmental Toxicity Studies

> Adequate data are available for malathion for evaluation of developmental toxicity in rats and rabbits. In rabbits, developmental effects (slightly increased incidence of mean resorption sites per dam) were noted at 50 mg/kg/day where maternal toxicity was also observed. No developmental effects were noted in rats at the highest dose tested (800 mg/kg/day) while maternal toxicity (cholinergic signs and reduced mean body weights) were observed in both species at this dose.

Executive summaries of developmental toxicity studies in rats and rabbits are as follows.

Malathion

In a **developmental toxicity study in rats (MRID 41160901)**, Malathion (94%) was administered by daily oral gavage to groups of 25 pregnant Sprague-Dailey dams on days 6 through 15 of gestation at dose levels of 0, 200, 400 or 800 mg/kg/day. No treatment-related mortalities occurred during the study. Clinical signs of toxicity were observed only at 800 mg/kg/day, consisting of urine stained abnormal fur in 5/25 dams and chromodacryorrhea and chromorhinorrhea in 1 dam. The maternal NOAEL is 400 mg/kg/day and the maternal LOAEL is 800 mg/kg/day based on reduced mean body weight gains and reduced mean food consumption during the period of treatment with Reference: National Cancer Institute. 1979. Bioassay of malaoxon for possible carcinogenicity, CAS No. 1634-88-2. Technical Report Series, No. 135, National Cancer Institute, Bethesda, MD. NCI-CG-TR-135. Assay performed at Gulf South Research Institute, New Iberia. LA.

Also see Huff et al., on the National Toxicology Program (NTP) reevaluation of malathion and malaoxon National Cancer Institute (NCI) rat carcinogenicity studies (Environ. Res. 37: 154-173, 1985) and Memorandum of E. McConnell, D.V.M. to J. Moore, D.V.M., June 14, 1984 with attached Summary Minutes of the NTP's Board of scientific counselors review of malathion malathion (especially days 6-12 of gestation). The developmental toxicity NOAEL is $\geq$ 800 mg/kg/day, the highest dose level tested. The developmental toxicity LOAEL is > 800 mg/kg/day since no adverse developmental effects were observed at any dose level in this study. The study is classified as **ACCEPTABLE**.

In a **developmental toxicity study in rabbits (MRID 00152569)**, Malathion (92.4%) was administered by daily oral gavage to groups of 20 pregnant New Zealand white does on days 6 through 18 of gestation at dose levels of 0, 25, 50 or 100 mg/kg/day. Anorexia and soft stools may have occurred at slightly higher incidence in the 100 mg/kg/day animals. The maternal NOAEL is 25 mg/kg/day and the maternal LOAEL is 50 mg/kg/day based on reduced mean body weight gains during days 6-18 of gestation (period of treatment with malathion). The developmental toxicity NOAEL is 25 mg/kg/day and the developmental toxicity LOAEL is 50 mg/kg/day based on an increased incidence of mean resorption sites per doe. The study is classified as **ACCEPTABLE**.

In a **range-finding study in rabbits (MRID 00152569)**, pregnant New Zealand white rabbits (5/group) received oral administration of Malathion (92.4%) in corn oil at doses of 0, 25, 50, 100, 200, or 400 mg/kg/day on Gestation Days (GD) 6-18. No mortalities or clinical signs were observed at 25, 50 or 100 mg/kg/day. At 200 mg/kg/day, 2 does died, 1 on GD 11 (5 days after dosing) and another on GD 17 (11 days after dosing). At 400 mg/kg/day, 4 does died, 1 on GD 7, 1 on GD 8 and 2 on GD 9. Cholinergic signs of toxicity seen at 200 and 400 mg/kg/day included tremors, decreased activity and salivation. External examinations of the fetuses did not indicate any gross abnormalities. For Maternal Toxicity, the NOEL was 100 mg/kg/day and the LOEL was 200 mg/kg/day based on mortality and clinical signs.

3.5 Reproduction Study

Malathion

> Malathion did not induce reproductive toxicity in rats at the highest dose tested. Although the offspring NOAEL was lower than the parental systemic NOAEL, pup body weight decrements were primarily observed, not at birth but during lactation, at postnatal day 21. At that time, young rats consume approximately twice the diet per unit body weight than do adult rats. Thus, the test substance intake by these animals is likely to be more than double the adult intake because of the ingestion of the test material both via the milk (lactation) and food.

An executive summary of a two-generation reproduction study in rats is as follows.

In a **two-generation reproduction study in rats (MRID 41583401)**, malathion (94.0% purity) was administered continuously in the diet for 2 successive generations to groups of 25 male and 25 female Sprague-Dawley rats at dose levels of 0, 550, 1700, 5000 or 7500 ppm (equivalent to 0, 43, 131, 394 or 612 mg/kg/day in males and 0, 51, 153, 451 or 703 mg/kg/day in females). Following 63 days of treatment (at about 105 days of age), of males and females were mated (1:1) to produce the F1A litters. Two weeks after weaning, OF males and females were again mated to produce the F1B litters. One male and one female F1B pup/litter were randomly selected to be F1 parents. Following 79 days of treatment, F1 males and females were mated, as before, to produce F2 and F2B litters. No treatment-related mortality or clinical signs of toxicity were observed in the F0 or F1 parental animals at any dose level.

The parental toxicity NOAEL is 5000 ppm (394 mg/kg/day in males and 451 mg/kg/day in females) and the parental toxicity LOAEL is 7500 ppm (612 mg/kg/day in males and 703 mg/kg/day in females) based on decreased body weights in F0 females during gestation and lactation and on decreased body weights in F1 males and females during the pre-mating period. The developmental toxicity NOAEL is 1700 ppm (131 mg/kg/day in males and 153 mg/kg/day in females) and the developmental toxicity LOAEL is 5000 ppm (394 mg/kg/day in males and 451 mg/kg/day in females) based on decreased pup body weights during the lactation period in F1A and F2B pups. The reproductive toxicity NOAEL is ≥ 7500 ppm (612 mg/kg/day in males and 703 mg/kg/day in females). The reproductive toxicity LOAEL is >7500 (612 mg/kg/day in males and 703 mg/kg/day in females). No reproductive toxicity was observed in this study. The study is classified as **ACCEPTABLE**.

3.6 Mutagenicity Studies

> Results of three guideline genetic toxicology studies with malathion indicate that the test material did not cause gene mutations in bacteria or unscheduled DNA synthesis (UDS) in cultured rat hepatocytes. Similarly, malathion was neither clastogenic nor aneugenic up to doses that showed clear cytotoxicity for the target tissue *in vivo*. Studies from the open literature indicated that malathion was positive both *in vitro* and *in vivo*. However, there

are uncertainties regarding the relevance of these findings to a possible mutagenic mode of action for malathion since positive results from both *in vivo* and *in vitro* studies were seen only at cytotoxic doses and/or the types of induced aberrations were asymmetric and, therefore, not consistent with cell survival. Questions also arise regarding the purity of the test material. Nevertheless, malathion was shown to be weakly reactive with DNA and does contain a structure that suggests electrophilicity. The Committee concluded, however, that the weight of the evidence neither supports a mutagenic hazard nor a role for mutagenicity in the carcinogenicity associated with malathion.

The overall assessment of studies from the open literature indicating positive clastogenicity should be intrepreted with caution. While 5 of 7 *in vivo* bone marrow studies were reported positive by Flessel *et al.*, (1993), evidence of structural chromosome damage was either accompanied by cytotoxicity (i.e., significantly reduced mitotic indices or increased cell cycle delay) or asymmetrical structural aberrations (i.e., chromatid and chromosome breaks and exchanges). Questions also arise regarding the purity of the test agent. A similar observation regarding cytotoxicity and the induction of unstable aberrations, which generally lead to death and hence do not directly contribute to carcinogenesis, can also be made for the 5 of 6 positive *in vitro* cytogenetic assays. Weak but positive results were shown for sister chromatid exchange induction at high, cytotoxic doses (Galloway et al., 1987) and for methylation in a submitted metabolism study (MRID 41367701). No assays with germinal cells have been submitted to the Agency. However, malathion was negative in *Drosophila melanogaster* sex linked recessive lethal assays, mouse dominant lethal assays and spermatogonia and/or spermatocyte cytogenetic assays. An adverse heritable effect has not been suggested for malathion.

No mutagenicity studies have been submitted to the Agency on the major metabolite of malathion, malaoxon. The consensus opinion from reviews of the open literature is that malaoxon is not mutagenic in bacteria but is a confirmed positive without S9 activation in the mouse lymphoma assay forward gene mutation assay. Malaoxon was not clastogenic in cultured Chinese hamster ovary (CHO) cells; however, the findings from the mouse lymphoma assay suggest that malaoxon may induce both gene mutations and chromosome aberrations. Malaoxon has a structure similar to malathion and, therefore, concerns for possible electrophilicity also apply to malaoxon. Nevertheless, malaoxon is not carcinogenic in males or females Fischer 344 rats.

Summaries of acceptable mutagenicity studies.

In a **Salmonella typhimurium/Escherichia coli reverse gene mutation assay (MRID 40939302)**, malathion (95.4%) was negative in independent trials up to the highest dose tested (5000 ug/plate) with or without S9 activation.

In an **in vivo bone marrow cytogenetic assay (MRID 41451201)**, malathion (94% a.i.) was negative following the single oral gavage administration of 500-2000 mg/kg to male and female Sprague-Dawley rats. A dose-related reduction in mitotic indices (MIs) was seen in the females of all treatment groups at 24 hours. Reduced MIs were also recorded for high-dose males and females at 48 hours.

In an **in vitro primary rat hepatocytes unscheduled DNA syntheses (UDS) assay (MRID 41389301)**, malathion (94% a.i.) was negative up to cytotoxic levels ($\geq$ 0.12 uL/mL; = 150 ug/mL).

3.7 Neurotoxicity Studies

> Available neurotoxicity studies are adequate to satisfy the guideline requirements. However, the Agency has recently issued FR42945 (August 6, 1999) requiring registrants of neurotoxic pesticides to conduct acute, subchronic, and developmental neurotoxicity studies. Thus, a developmental neurotoxicity study for malathion is required under this Data Call-in program.
>
> The acute delayed neurotoxicity study in the hen did not reveal any treatment-related findings at gross necropsy nor histopathological examination in hens. In acute and subchronic neurotoxicity studies, neurotoxic effects were observed which included clinical signs, inhibition of brain, plasma, or RBC cholinesterase activity.

Executive summaries for acute oral delayed neurotoxicity study in hen, acute and subchronic oral neurotoxicity studies in rats are as follows.

In an **acute delayed neurotoxicity study in hens (MRID 40939301)**, technical grade malathion (93.6% purity) was administered in a single oral dose by gavage to 60 mature White Leghorn hens at a dose level of 1007.5 mg/kg (1.3 x the oral LD50 of 775 mg/kg). The hens were atropinized previously with 10 mg/kg of atropine sulfate IM and ½, 1, 3 and 5 hours post-dosing with 30 mg/kg IM. Twenty-one days later, survivors were again given malathion at a dose level of 852.5 mg/kg (1.1 x the LD50). The birds were atropinized as before. Twenty-one days later (42 days after the first dose), the surviving hens were sacrificed. Fifteen negative control hens were treated similarly but were given tap water, rather than malathion, on days 0 and 21. In this study, hens treated with malathion did not exhibit any evidence of acute delayed neurotoxicity. This study is classified **ACCEPTABLE**.

In an **acute neurotoxicity in rats (MRID 43146701)**, Malathion was evaluated for acute neurotoxicity, including cholinesterase inhibition, using Sprague-Dailey rats in groups of 27 rats/sex following single oral gavage dosages of 0, 500, 1000 or 2000 mg/kg in corn oil. FOB, locomotor activity, histopathology and cholinesterase assays were performed at pretest, peak effect (15 minutes post-dosing), day 7 and day 14. Treatment-related clinical signs were observed at all doses, being most definitive at the 2000 mg/kg dose level. Among FOB parameters (home cage, handling, open field, sensory, neuromuscular and physiological observations) and locomotor activity, there were no remarkable treatment-related effects except a possible decreased motor activity among rats at the 2000 mg/kg level.

For rats of both sexes, brain cholinesterase NOAEL was the highest dose tested, 2000 mg/kg. Among females, plasma cholinesterase was possibly inhibited (ranging 11-48%) at all doses on days 0, 7 and 15, being statistically significant only at 500 mg/kg on day 7. A dose response was not evident. High variability in assay results, coupled with small numbers of animals (5/sex/group) at given time points render a conclusion as to NOAEL/LOAEL difficult. In males, no effect was observed on plasma cholinesterase. Concerning erythrocyte cholinesterase, among females, statistically significant inhibition of 39% and non-significant inhibition of 34%, respectively, at 2000 and 1000 mg/kg on day 7 support an effect in females, where LOAEL/NOAEL = 2000/1000 mg/kg and possibly 1000/500 mg/kg. In males there were no statistically significant inhibitions of this enzyme, though there was a 40% non-significant inhibition at dy 7 at 2000 mg/kg. The study is classified **ACCEPTABLE**.

In a **subchronic neurotoxicity study (MRID 43269501)**, technical malathion (96.4% a.i.) was administered continuously in the diet for 90 days to groups of 25 male and female Sprague-Dawley rats at dose levels of 0, 50, 5000 or 20,000 ppm (equivalent to 0, 4, 352 and 1486 mg/kg/day for males an 0, 4, 395 or 1575 mg/kg/day for females). The rats were subjected to neurotoxicity assessment at pretest, weeks 3, 7 and 12. Plasma, erythrocyte and brain region cholinesterase determinations were performed on 5 rats/sex/group one week prior to study initiation and during weeks 3, 7 and 13. Definite effects were noted in the high dose group only, which included cholinergic signs and decreased body weight gain. Among neurotoxicity parameters (FOB and motor activity) there were no effects. Hence, LOAEL is 1486 (males), 1575 (females) mg/kg/day. The NOAEL is 352 (males) 395 (females) mg/kg/day. For cholinesterase inhibition, plasma cholinesterase (males 12-20%, females 15-30%, erythrocyte cholinesterase (males 49-61% and females 49-53%) and brain (i.e., cortex 12-20% in females) were inhibited at 352 or 395 mg/kg/day, respectively. Higher levels of cholinesterase inhibition were noted for the high dose group and male brain (i.e. mid-brain 24%). The LOAEL is 352 (males), 395 (females) mg/kg/day based on plasma and erythrocyte cholinesterase, and 395 (females) mg/kg/day based on brain cholinesterase. The NOAEL is 4 mg/kg/day based on plasma and erythrocyte cholinesterase in both sexes and brain cholinesterase in females. The study is classified as **ACCEPTABLE**.

3.8 Metabolism Studies

In the rat, malathion is excreted primarily in the urine (80-90%) in the first 24 hours following exposure, with lesser amounts excreted in the feces. At 72 hours, the highest concentration of radioactivity was observed in the liver, but less than 0.3% of the administered radioactivity was present in that organ. Radioactivity did not bioaccumulate in any of the organ/tissues analyzed. Although eight radiolabeled metabolites were

> observed in urine, greater than 80% of the radioactivity in urine was represented by the diacid (DCA) and monoacid (MCA) metabolites. The remaining radiolabeled metabolites were identified as components of "peak A" and "peak B". It was determined that between 4 and 6% of the administered dose was converted to malaoxon, the active cholinesterase inhibiting metabolite of malathion.

The executive summary of a metabolism study in rats is as follows.

In a **metabolism study in rats (MRID 41367701)**, single doses of radiolabeled 14C-malathion (98% purity) were administered by oral gavage to groups of 5 male and 5 female Sprague-Dawley rats at dose levels of 40 mg/kg, 800 mg/kg and 40 mg/kg following 15 days of daily oral gavage of non-radio labeled malathion (94.6%) at a dose level of 40 mg/kg/day. The rats were then placed in metabolism cages and urine and feces were collected for 72 hours for determination of excretion of radioactivity and analysis of biotransformation products. At 72 hours, the animals were sacrificed and major organs/tissues were collected, weighed and analyzed for radioactivity.

More than 90% of the radioactivity in the 40 mg/kg dose was excreted within 72 hours, with most excretion occurring in the first 24 hours. Approximately 80-90% of the administered radioactivity was excreted in the urine. Only minor differences in urine/fecal excretion ratios were observed between animals given 40 mg/kg, 800 mg/kg and 40 mg/kg after 15 previous daily doses of malathion. At 72 hours, the highest concentration of radioactivity was observed in the liver, but less than 0.3% of the administered radioactivity was present in that organ. Radioactivity did not bioaccumulate in any of the organs/tissue analyzed. Although 8 radiolabeled metabolites were observed in urine, greater than 80% of the radioactivity in urine was represented by the diacid and monoacid metabolites. It was determined that between 4 and 6% of the administered dose was converted to malaoxon. The study is classified as **ACCEPTABLE**.

3.9 Dermal Absorption

> No guideline dermal penetration study has been submitted to the Agency in support of reregistration. There is a published literature data available with human volunteers (MRID 05003588). Based on the information, the HIARC concluded the dermal absorption rate to be about 10%, and recommended that a dermal absorption factor of 10% be used for converting oral dosing to dermal dosing. The 10% dermal absorption factor is supported by comparison of NOAELs and LOAELs in the oral developmental toxicity study and the 21-day dermal toxicity study in the same species (rabbits).

In a **dermal absorption study on humans (MRID 05003588)**, ^{14}C-radiolabeled malathion (dissolved in acetone) was applied to a 13 sq cm circular area on the ventral surface of the forearms of 7 subjects at a rate of 4 μgm/sq cm. The skin sites were not protected. All urine was collected for 5 days and assayed for radioactivity in a liquid scintillation counter. Dermal penetration of malathion through the skin was estimated by calculating the total amount of radioactivity excreted in the urine in 5 days.

A mean of 7.84% ± 2.71% (SD) of the applied dose of radioactivity was recovered in the 5 day urine, indicating a dermal absorption rate of approximately 5% to 10% over a 5 day period. This study is classified **acceptable/nonguideline**.

4.0 CLASSIFICATION OF CARCINOGENIC POTENTIAL

The Health Effects Division's Cancer Assessment Review Committee (CARC) has met to review the carcinogenic potential of malathion on September 24, October 8, and October 15, 1997, June 10, 1998, February 24, and June 23, 1999. The Committee reviewed the following studies: 1) Carcinogenicity study in B6C3F1 mice; 2) Combined chronic toxicity/carcinogenicity study in Fischer 344 rats with malathion; and 3) the Combined chronic toxicity/carcinogenicity study with malaoxon, the active cholinesterase inhibiting metabolite of malathion in F344 rats. Relevant subchronic, chronic and mutagenicity studies were also reviewed at these meetings, as well as the results of the studies conducted with malathion and/or malaoxon (during 1978-80) by the National Cancer Institute/National Toxicology Program (NCI/NTP), and a Pathology Working Group (PWG) report on the female Fischer 344 rat liver tumors. On April 12, 2000, the CARC met to evaluate: 1) a new Pathology Working Group (PWG) report on the female Fischer 344 rat liver tumors; 2) two issues raised by Dr. Dementi regarding the evaluation of malathion (mononuclear cell leukemia in Fischer 344 male rats and oral tumors in Fischer 344 female rats); 3) the March 29, 2000 letter from Jellinek, Schwartz & Connally, Inc. to Patricia Moe, *Re: Comments on EPA's Risk Assessments for Malathion*; 4) discuss the weight of evidence and cancer classification for malathion based on the previously listed information.

The Committee concluded that there is evidence of carcinogenicity in both sexes of mice at the two highest dose levels of malathion tested which were considered excessive. There is no evidence of carcinogenicity in male or female mice at the lower doses. Evidence for carcinogenicity in mice is demonstrated by the presence of liver tumors in both sexes. The Committee further concluded that there is evidence of carcinogenicity for malathion in female rats at the highest dose which was considered excessive. The Committee determined that the oral (females at 6000 and 12,000 ppm) and nasal tumors (females at 6000 and 12,000 ppm and males at 12,000 ppm) could not be distinguished as either treatment-related or of random occurrence.

The Committee also concluded that the following tumors are NOT treatment related:
Male rats - 1) **thyroid gland (follicular cell)** - there was neither statistical (other than a positive trend for combined adenomas and carcinomas) nor biological significance for any tumor type. Although there was no evidence that the above tumors are treatment related in rats at any dose level, the potential for tumor induction may have been compromised by competing toxicity, particularly at 6000 ppm and 12000 ppm, where mortality was 74% and 100%, respectively. There is, however, no evidence to either support or refute this supposition.
2) **thyroid gland (c-cell)** there was neither statistical (other that carcinomas in the 500 ppm

group) nor biological significance, there was no dose-response relationship, and the combined tumor incidences in treated groups were comparable to those seen in the concurrent control group.
3) **testes (interstitial cell)** - tumor incidences of this nonfatal tumor were approaching 100% in all groups including controls, and positive statistical significance was considered to be an artifact in the Peto's Prevalence Analyses due to high mortality rather than biologically meaningful.
4) **liver** - there was neither statistical nor biological significance and there was no dose-response relationship. Although there was no evidence that the above tumors are treatment related in rats at any dose level, the potential for tumor induction may have been compromised by competing toxicity, particularly at 6000 ppm and 12000 ppm, where mortality was 74% and 100%, respectively. There is, however, no evidence to either support or refute this supposition.
5) **mononuclear cell leukemia** (MCL) - this tumor occurs commonly in Fischer rats and the incidences were within historical control ranges, there was no statistical significance at any dose, there was no dose response, there was no indication of early onset or increased incidence. Further more, attributing the cause of death to MCL is subjective and not a reliable indicator of increased severity this tumor.
<u>**Female rats**</u> - 6) **pituitary gland (par distalis)** - the tumor incidences and types in treated groups were comparable to those seen in the concurrent control group, there was neither statistical nor biological significance, and there was no dose-response relationship.
7) **uterus (various types)** - the individual tumor incidences were low, the tumor incidences and types in treated groups were comparable to those seen in the concurrent control group, there was neither statistical nor biological significance, and there was no dose-response relationship.

Results of the guideline genetic toxicology studies with malathion indicate that the test material did not cause gene mutations in bacteria or UDS in cultured rat hepatocytes. Similarly, malathion was neither clastogenic nor aneugenic up to doses that showed clear cytotoxicity for the target tissue *in vivo*. The CARC included that *in vitro* and *in vivo* findings from the open literature should be interpreted with caution since positive results were seen at cytotoxic doses and/or the types of induced aberrations were asymmetric and, therefore, not consistent with cell survival. The question of test material also was an issue. Although the structure of malathion suggests electrophilicity, **the Committee concluded that the weight of the evidence supports neither a mutagenic hazard nor a role for mutagenicity in the carcinogenicity associated with malathion.**

Malaoxon, the active cholinesterase inhibiting metabolite of malathion, was not carcinogenic in male or female rats when tested at doses that were judged to be adequate to assess its carcinogenic potential. MCL was not considered to be treatment related since: (1) statistical significance was seen only in males at a dose that was determined to be excessive, (2) there was no dose-response, and (3) the incidences were within the historical control range of the testing laboratory. Malaoxon was non-mutagenic in bacteria, was not clastogenic in cultured Chinese hamster ovary (CHO) cells, but did produce positive results without metabolic activation in the mouse lymphoma assay. Malaoxon caused sister chromatid exchanges in CHO cells in the absence of metabolic activation. Malaoxon has a structure similar to malathion; hence, the possibility of electrophilicity may also apply, despite the evidence of no carcinogenicity.

In accordance with the EPA *Proposed Guidelines for Carcinogen Risk Assessment* (July 1999), the Committee at the April 12, 2000 meeting, classified malathion as **'suggestive evidence of carcinogenicity but not sufficient to assess human carcinogenic potential"** by all routes of exposure. This classification was based on the following factors:

(i) occurrence of liver tumors in male and female B6C3F1 mice and in female Fischer 344 rats only at excessive doses (statistically significant and outside historical control);
(ii) the presence of a few rare tumors, oral palate mucosa in females and nasal respiratory epithelium in male and female Fischer 344 rats. With the exception of one nasal and one oral tumor in female rats, all other tumor types were determined to occur at excessive doses or were unrelated to treatment with malathion. These tumors can not be distinguished as either treatment related or due to random occurrence;
(iii) the evidence for mutagenicity is not supportive of a mutagenic concern in carcinogenicity; and
(iv) malaoxon, a structurally related chemical, is not carcinogenic in male or female Fischer 344 rats.

Quantitative risk assessment for carcinogenicity is not required since the Committee classified malathion as having suggestive evidence for cancer. A cancer dose-response assessment, e.g. a low dose linear extrapolation model, is not indicated for pesticides in the "suggestive" category.

5.0 HAZARD ENDPOINT SELECTION

The HED HIARC evaluated the toxicological database of malathion, established Reference Dose (RfD), and selected the toxicological endpoints for acute dietary as well as occupational and residential exposure risk assessment (HIARC reports, HED DOC. No. 012440, 013032, and 013820). The doses and toxicological endpoints selected on malathion for various exposure scenarios are summarized below.

Malathion

EXPOSURE SCENARIO	DOSE (mg/kg/day)	ENDPOINT	STUDY
Acute Dietary (single day)	NOAEL=50	Maternal toxicity	Range Finding and Main Developmental Toxicity Studies - Rabbits
	UF=100 (10X10)	**Acute RfD = 0.5 mg/kg/day**	
	FQPA Safety Factor Removed (1x)	**Acute PAD = 0.5 mg/kg/day**	
Chronic Dietary	NOAEL=2.4	Inhibition of plasma cholinesterase activity	Combined Chronic Toxicity/ Carcinogenicity Study in the Rat
	UF=100 (10X10)	**Chronic RfD = 0.024 mg/kg/day**	
	FQPA Safety Factor Removed (1x)	**Chronic PAD = 0.024mg/kg/day**	
Carcinogenicity	Malathion is classified as "**suggestive evidence of carcinogenicity but not sufficient to assess human carcinogenic potential**".		
Short-Term (Dermal) 1-7 days	NOAEL=50	Inhibition of plasma, RBC, and brain cholinesterase activity	21-day Dermal Study in the Rabbit
	UF=100 (10X10) for occupational and non-occupational populations (FQPA Safety Factor Removed (1x)		
Intermediate-term (Dermal) 1 week to several months	NOAEL = 50	Inhibition of plasma, RBC, and brain cholinesterase activity	21-day Dermal Study in the Rabbit
	UF=100 (10X10) for occupational and non-occupational populations (FQPA Safety Factor Removed (1x))		
Long-Term (Dermal) >180 days	Oral NOAEL = 2.4	Inhibition of plasma cholinesterase activity	Combined Chronic Toxicity/ Carcinogenicity Study in the Rat
	UF=100 (10X10) for occupational and non-occupational populations (FQPA Safety Factor Removed (1x)) dermal absorption = 10%		
Inhalation (Short, Intermediate, and Long Term)	LOAEL = 25.8 mg/kg/day The inhalation LOAEL of 0.1 mg/L was converted to 25.8 mg/kg/day.	Inhibition of plasma and RBC cholinesterase activity and histopathology in respiratory epithelium	90-Day Inhalation Study in the Rat
	UF = 1000 (10x10x10) for the lack of a NOAEL and the severity of the nasal lesions observed in the two-week range finding study (100% inhalation absorption) for all occupational and non-occupational populations which include infants and children (FQPA Safety Factor Removed (1x)).		

The inhalation LOAEL of 0.1 mg/L was converted to an oral equivalent dose of 25.8 mg/kg/day for use in MOE calculations based on HED's route-to-route extrapolation methodology (J. Whalen and H. Pettigrew, October 10, 1998).

5.1 Acute Reference Dose (RfD)

For acute dietary assessment, the endpoint was selected from a range-finding developmental toxicity and the main study in rabbits based on a weight-of-the-evidence approach as well as pertinent information from other studies. The acute reference dose (Acute RfD) is 0.5 mg/kg/day based on a NOAEL of 50 mg/kg/day and an uncertainty factor of 100 (10x for inter-species extrapolation and 10x for intra-species variation). The FQPA safety factor (10x) is removed based on the following factors: (i) developmental toxicity studies showed no increased sensitivity in fetuses as compared to maternal animals following *in utero* exposures in rats and rabbits; (ii) a two-generation reproduction toxicity study in rats showed no increased sensitivity in pups when comparted to adults; (iii) neuropathology and OPDIN were negative; and (iv) the toxicology data base is complete and there are no significant data gaps at this time.

5.2 Chronic Dietary Reference Dose (RfD)

The Reference Dose (RfD) for chronic dietary exposure is 0.024 mg/kg/day based on a NOAEL of 2.4 mg/kg/day in a combined chronic toxicity/carcinogenicity study in rats (MRID 43942901) where significant inhibition of plasma cholinesterase activity was observed at 29 mg/kg/day (LOAEL). For chronic dietary risk assessment, **a UF of 100 is adequate** for the protection of the general U.S. population including infants and children from chronic exposure to Malathion.

5.3 Occupational/Residential Exposure

5.3.1 Dermal Absorption

Dermal Absorption Factor: A dermal absorption factor of 10% should be used for converting oral dosing to dermal dosing.

5.3.2 Short and Intermediate Term Dermal

A 21-day dermal toxicity study was selected based on significant inhibition of plasma, red blood cell or brain cholinesterase activity at 300 mg/kg/day (LOAEL) and the NOAEL = 50 mg/kg/day. The selected dose/endpoint is supported by the NOAEL of 4 mg/kg/day established following oral exposure in the 13-week neurotoxicity study in rats when a dermal absorption factor of 10% is applied. In both studies (i.e. via both routes), the LAOEL was based on a common toxicological endpoint, inhibition of plasma, red blood cell and brain cholinesterase activity.

This risk assessment is required.

5.3.3 Long Term Dermal

Malathion

A combined chronic toxicity/carcinogenicity study in rats was selected where significant inhibition of plasma cholinesterase activity was observed at 29 mg/kg/day (LOAEL) and the NOAEL is 2.4 mg/kg/day. This dose and endpoint was used in establishing the RfD. Since an oral dose was selected, a dermal absorption rate of 10% should be used in dermal risk assessments.

This risk assessment is required.

5.3.4 Inhalation exposure (Any-Time Period)

The endpoint/doseof 25.8 mg/kg/day (LOAEL) is selected from a 90-day inhalation study in rats based on inhibition of plasma and red blood cell cholinesterase activity and histopathological lesions of the nasal cavity and larynx at the lowest concentration tested. Since this is the only inhalation study that is available in the toxicology data base, the LOAEL from this study will be used for Short-, Intermediate-and Chronic inhalation risk assessments.

The Margin of Exposure (MOE) of 1000 is required for Short-, Intermediate- and Long-Term inhalation exposures based on the results of a two-week range finding study (MRID 44554301) where there was a dose-related increase in the lesions of the nasal cavity (hyperplasia and respiratory epithelium) which was similar to the laryngeal and nasal cavity lesions seen in the subchronic study. The MOE of 1000 includes the conventional 100 and an additional 10 for the use of a LOEL and the severity of the nasal lesions. The HIARC also determined that a new inhalation study is required based on the results of the two-week range-finding study and the lack of a NOAEL for cholinesterase inhibition in the 90-day study (MRID 43266601).

5.4 Recommendation for Aggregate Exposure Risk Assessments

For aggregate exposure risk assessment, the MOEs derived for the oral, dermal and inhalation exposures may be combined to obtain a total MOE since a common toxicological endpoint (i.e., cholinesterase inhibition) was observed following exposure via these routes in oral, dermal and inhalation toxicity studies.

$$\frac{1}{\dfrac{1}{MOE_{Oral}} + \dfrac{1}{MOE_{Dermal}} + \dfrac{1}{MOE_{Inhalation}}}$$

5.5 Classification of Carcinogenic Potential

In accordance with the EPA *Proposed Guidelines for Carcinogen Risk Assessment* (July 1999), the Committee at the April 12, 2000 meeting, classified malathion as **'suggestive evidence of carcinogenicity but not sufficient to assess human carcinogenic potential."**

Quantitative risk assessment for carcinogenicity is not required.

6.0 FQPA CONSIDERATIONS

6.1 Special Sensitivity to Infants and Children

There is no indication of additional sensitivity to young rats or rabbits following pre-and/or postnatal exposure to Malathion in the developmental and reproductive toxicity studies.

6.2 Recommendation for a Developmental Neurotoxicity Study

The developmental neurotoxicity study is required under the Data Call-in program, FR42945, August 6, 1999.

6.3 FQPA Safety Factor Committee Recommendation

The Committee determined that for Malathion, the **10 x factor** to account for enhanced sensitivity of infants and children (as required by FQPA) **should be removed.** This conclusion was based on the following factors.

- (i) Developmental toxicity studies showed no increased sensitivity in fetuses as compared to maternal animals following *in utero* exposures in rats and rabbits.
- (ii) A two generation reproduction toxicity study in rats showed no increased sensitivity in pups when compared to adults.
- (iii) Neuropathology and OPDIN were negative.
- (iv) The toxicology data base is complete and there are no data gaps at this time.

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